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# Oil-tainting of fish, a laboratory test on salmon and saithe

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Per Olav Brandal, Otto Grahl-Nielsen, Tore Neppelberg, Karsten H. Palmork, Kjell Westrheim, Svein Wilhelmsen Institute of Marine Research, Directorate of Fisheries. P.O. Box 1870-72, 5011 Bergen-Nordnes

Norway

## INTRODUCTION

One of the consequences of oil pollution of the marine environment is tainting of fish by petroleum hydrocarbons which are assimilated into their tissue. The problem is of special importance concerning caught and cultured fish which are kept in seine-nets or other installations in coastal waters.

Some investigations have been carried out, see a review by McIntyre et al. 1, but there are still many unknown factors e.g.: which components of oil are responsible for the taint and what are the threshold levels, which connections are there between hydrocarbon concentration in water and tainting, how do different fish species respond, how fast do they respond and how long time do they need for depuration in clean water, etcetera.

The present investigation was conducted to study how a lean fish, saithe Gadus virens, and a fat fish, salmon Salmo salar, respond to pollution by crude oil. The experiment was thought to be

To whom correspondence should be addressed.

a simulation of the following situation: Oil is spilt in an accident, the oil slicks drift for a couple of days before they reach and contaminate an installation where caught or cultured fish are present without any possibility to avoid contamination. Such a situation resently occured on the Norwegian coast <sup>2</sup>, and migt very well happen again.

## EXPERIMENTAL

Ekofisk crude, 35 1, was subjected to weathering on a 2 square meter surface of a 1 cubic meter vessel through which 19 litres seawater per minute was flowing. After 3 days the remaining oil was transferred to another vessel with 25 cubic meter capacity and a through-flow of 85 - 90 litres per minute. The temperature of the water was coustant at  $8.5 \,^{\circ}$ C. The vessel contained 100 saithe <u>Gadus virens</u> of 3 - 400 grams weight and 50 salmon Salmo salar of approximately 100 grams weight.

Samples were taken at intervals of the water from the drain tube in the bottom of the vessel, of the oil on the surface of the water and of the fish. When collecting the fish a special boom arrangement was used to clear parts of the surface of the vessel to avoid direct contact of the fish with the oil.

The experiment proceeded in this manner for 68 days, the fish were not fed during this time, Thereafter the reamining fish were transferred to a vessel with clean water and they were fed regularly.

The water was analysed quantitatively by gas chromatography, and the oil was analysed by gas chromatography coupled to mass spectrometry according to Grahl-Nielsen et al.<sup>2</sup>.

The fish was analysed organoleptically and chemically. For organoleptical analysis the fish were wrapped individually in aluminium foil and steam boiled in separate pans. This preparation was expected to give the least disapperance of taste components. A taste panel of selected persons compared the treated fish with con trols.

Liver and muscle of the fish were analysed for hydrocarbons by gas chromatography coupled with mass spectrometry  $\frac{2}{2}$ .

#### RESULTS AND DISCUSSION

The water contained an average of 40 - 50 micrograms hydrocarbons (µg) per litre. There was a trend, although not very significant, of lower concentration towards the end of the experiment. A significant change, however, was observed in the quality of the hydrocarbons in the water: In the beginning volatile hydrocarbons dominated, later the contents shifted towards less volatile components, and the chromatograms appeared to be more similar to those of the oil on the surface. This implies that in the beginning the hydrocarbons are brought into the water by dissolution, the most volatile components are also the most soluble, while the hydrocarbons found in the water at a later stage when most of the volatile components have disappeared from the oil on the surface, are transferred into the water as oil-in-water emulsion.

The pollution of the vessel by oil gave no increase in mortality of the fish, as a matter of fact, no significant change in the behaviour of the fish could be observed after the oil was transferred to the vessel.

Oil tainting of the salmon was first discovered after 4 days, the tainting was obvious after 6 days and increased further to a maximum after 8 days. Five days later the taint had decreased significantly, but it was still present approximately on the same level as when first detected after 4 days. After 15 days remaining taint was questionable and after 22 days it had definetely disappeared, the salmon from the polluted vessel were at this stage identical with the controls. The response of the saithe was completely different from that of the salmon: the only tendency of taint was detected after 22 days, even this was not very significant. After 28 days the test fish could not be distinguished from the controls.

In the chemical analysis of the fish samples the search was concentrated on aromatic hydrocarbons. The only components found in significant amounts were benzene derivatives and naphthalene derivatives. Benzene derivatives are too volatile for proper quantification, but they appear to be present in amounts simular to the naphthalene derivatives. The quantification was then based on naphthalene, mono-, di- and trimethylnaphthalene and the values discussed below are in micrograms per gram fat with micrograms per gram wet weight in parantheses.

After only 7 hours significant amounts of naphthalenes had been taken up in the liver of the saithe i.e. from a background level of 0.06 (0.03)  $\mu$ g/g before the experiments started to 5.4 (2.1)  $\mu$ g/g. The amounts in the liver increased to around 30 (10)  $\mu$ g/g after 4 days, remained at that level for about eleven days then decreased slowly while the oil was still on the surface. After 68 days with oil on the surface and another 24 days in clean water the naphthalenes concentration in the liver was the same as before commencement of the experiment.

The contents of naphthalenes in the liver of the salmon followed a very simular pattern, but because of the smaller size of the livers there was a higher degree of incertainty in the quantification. During the first 7 hours the contents rose from 11 (0.2)  $\mu$ g/g to 42 (0.4)  $\mu$ g/g further increasing to 2 - 300 (2-3)  $\mu$ g/g after 6 - 8 days, and therafter decreasing while oil still was on the surface.

With relation to the organoleptic analysis, the naphthalene contents in the muscles are of greater interest. The concentrations was now determined only relative to wet weight i.e  $\mu$ g per gram. Here also the maximum was reached after 6 - 8 days but the level was significantly higher, with approximately 0.5  $\mu$ g/g in the salmon and 0.09  $\mu$ g/g in the saithe.

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After 39 days both saithe and salmon was depurated to approximately the same level,  $0.03 \ \mu g/g$  in saithe and  $0.02 \ \mu g/g$ in salmon. For saithe the contents ultimately dropped to  $0.005 \ \mu g/g$  after 24 days in clean water.

There was a striking connection between the contents of naphthalenes and the taint of the salmon. Both were increasing, reaching a maximum and decreasing concurrently. This does not necessarily mean that the naphthalenes are responsible for the taint, but at least the responsible components belong to the same fraction of the oil as the naphthalenes, i.e. with about the same volatility/solubility as the naphthalenes.

If the naphthalene content should be indicative of tainting the results suggest that the contents must be above approximately 0.3  $\mu$ g per gram muscle. The concentrations in the saithe never reached more than about 0.1  $\mu$ g/g. The trace of tainting on the saithe which was observed after 22 days must therefore, if noteworthy, be due to some other component of oil or metabolites thereof.

# SUMMARY

In a simulated oil spill 35 litres of Ekofisk crude were kept on the surface of a vessel with sea-water flowing through. The water obtained a concentration of 40 - 50 micrograms hydrocarbons per litre. From this water saithe and salmon within hours took up and accumulated in their liver and muscles benzene and naphthalene derivatives. The salmon was tainted after 4 - 8 days either from these components or metabolites thereof or from other components from the same fraction of the crude oil. The saithe was not tainted accordingly. Depuration of the taint and of the hydrocarbons started after 8 days while the remaining oil was still on the surface.

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